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## (54) A novel-beta1- 6 N-acetylglucosaminyltransferase, its acceptor molecule, leukosialin, and a method for cloning proteins having enzymatic activity.

(57) The present invention provides a novel β1—6 *N*-acetylglucosaminyltransferase, which forms core 2 oligosaccharide structures in *O*-glycans, and a novel acceptor molecule, leukosialin, CD43, for core 2 β1—6 *N*-acetylglucosaminyltransferase activity. The amino acid sequences and nucleic acid sequences encoding these molecules, as well as active fragments thereof, also are disclosed. A method for isolating nucleic acid sequences encoding proteins having enzymatic activity is disclosed, using CHO cells that support replication of plasmid vectors having a polyoma virus origin of replication. A method to obtain a suitable cell line that expresses an acceptor molecule also is disclosed.

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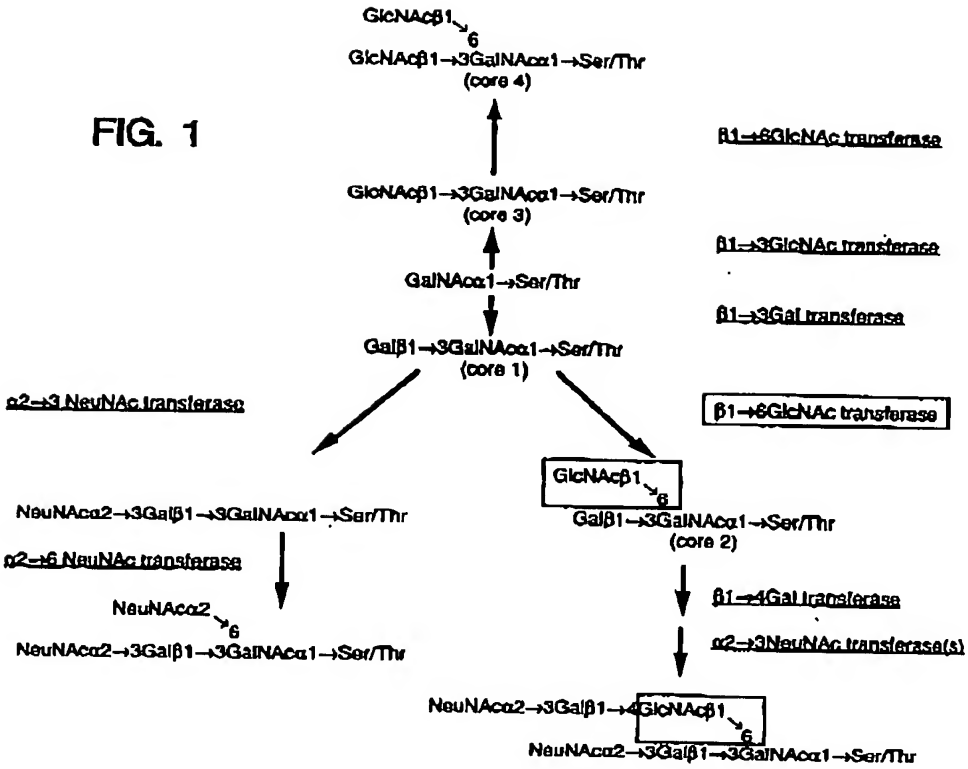
FIG. 1


GlcNAcβ1→6
GlcNAcβ1→3GalNAcα1→Ser/Thr
(core 4)
β1→6GlcNAc transferase
GlcNAcβ1→3GalNAcα1→Ser/Thr
(core 3)
β1→3GlcNAc transferase
GalNAcα1→Ser/Thr
β1→3Gal transferase
Galβ1→3GalNAcα1→Ser/Thr
(core 1)
α2→3 NeuNAc transferase
β1→6GlcNAc transferase
GlcNAcβ1→6
NeuNAcα2→3Galβ1→3GalNAcα1→Ser/Thr
Galβ1→3GalNAcα1→Ser/Thr
(core 2)
α2→6 NeuNAc transferase
β1→4Gal transferase
NeuNAcα2→6
NeuNAcα2→3Galβ1→3GalNAcα1→Ser/Thr
α2→3NeuNAc transferase(s)
NeuNAcα2→3Galβ1→4GlcNAcβ1→6
NeuNAcα2→3Galβ1→3GalNAcα1→Ser/Thr

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## BACKGROUND OF THE INVENTION

### FIELD OF THE INVENTION

This invention relates generally to the fields of biochemistry and molecular biology and more specifically to a novel human enzyme, UDP-GlcNAc:Gal$\beta$1→3GalNAc (GlcNAc to GalNAc) $\beta$1→6 *N*-acetylglucosaminyltransferase (core 2 $\beta$1→6 *N*-acetylglucosaminyltransferase; C2GnT), and to a novel acceptor molecule, leukosialin, CD43, for core 2 $\beta$1→6 *N*-acetylglucosaminyltransferase action. The invention additionally relates to DNA sequences encoding core 2 $\beta$1→6 *N*-acetylglucosaminyltransferase and leukosialin, to vectors containing a C2GnT DNA sequence or a leukosialin DNA sequence, to recombinant host cells transformed with such vectors and to a method of transient expression cloning in CHO cells for identifying and isolating DNA sequences encoding specific proteins, using CHO cells expressing a suitable acceptor molecule.

### BACKGROUND INFORMATION

Most *O*-glycosidic oligosaccharides in mammalian glycoproteins are linked via *N*-acetylgalactosamine to the hydroxyl groups of serine or threonine. These *O*-glycans can be classified into 4 different groups depending on the nature of the core portion of the oligosaccharides (see Fig. 1). Although less well studied than *N*-glycans, *O*-glycans likely have important biological functions. Indeed, the presence of *O*-linked oligosaccharides with the core 2 branch, Gal$\beta$1→3(GlcNAc$\beta$1→6)GalNAc, has been demonstrated in many biological processes.

Piller et al., J. Biol. Chem 263:15146-15150 (1988) reported that human T-cell activation is associated with the conversion of core 1-based tetrasaccharides to core 2-based hexasaccharides on leukosialin, a major sialoglycoprotein present on human T lymphocytes (see also Fig. 1). A similar increase in hexasaccharides was observed in peripheral blood lymphocytes of patients suffering from T-cell leukemias (Saitoh et al., Blood 77:1491-1499 (1991)), myelogenous leukemias (Brockhausen et al., Cancer Res. 51:1257-1263 (1991)) and immunodeficiency due to AIDS and the Wiskott-Aldrich syndrome (Piller et al., J. Exp. Med. 173:1501-1510 (1991)). In these patients' lymphocytes, changes in the amount of hexasaccharides were caused by increased activity of either UDP-GlcNAc:Gal$\beta$1→3GalNAc (GlcNAc to GalNAc) 6-$\beta$-D-*N*-acetylglucosaminyltransferase (EC2.4.1.102) or core 2 $\beta$1→6 *N*-acetylglucosaminyltransferase (Williams et al., J. Biol. Chem. 255:11253-11261 (1980)). Increased activity of core 2 $\beta$1→6 *N*-acetylglucosaminyltransferase also was observed in metastatic murine tumor cell lines as compared to their parental, non-metastatic counterparts (Yousefi et al., J. Biol. Chem. 266:1772-1782 (1991)).

Increased complexity of the attached oligosaccharides increases the molecular weight of the glycoprotein. For example, leukosialin containing hexasaccharides has a molecular weight of ~135kDa, whereas leukosialin containing tetrasaccharides has a molecular weight of ~105kDa (Carlsson et al., J. Biol. Chem. 261:12779-12786 and 12787-12795 (1986)).

Fox et al., J. Immunol. 131:762-767 (1983) raised a monoclonal antibody, T305, against human T-lymphocytic leukemia cells. Sportsman et al., J. Immunol. 135:158-164 (1985) reported T305 binding was abolished by neuraminidase treatment, suggesting T305 binds to hexasaccharides. T305 specifically reacts with the high molecular weight form of leukosialin (Saitoh et al., supra, (1991)).

Previous studies indicated poly-*N*-acetyllactosamine repeats extend almost exclusively from the branch formed by the core 2 $\beta$1→6 *N*-acetylglucosaminyltransferase (Fukuda et al., J. Biol. Chem. 261:12796-12806 (1986)). Consistent with these results, Yousefi et al., supra, (1991) demonstrated that the core 2 enzyme in metastatic tumor cells regulates the level of poly-*N*-acetyllactosamine synthesis in *O*-linked oligosaccharides.

Poly-*N*-acetyllactosamines are subject to a variety of modifications, including the formation of the sialyl $Le^x$, NeuNAc$\alpha$2→3Gal$\beta$1→4(Fuc$\alpha$1→3)GlcNAc-, or the sialyl $Le^a$, NeuNAc$\alpha$2→3Gal$\beta$1→3 (Fuc$\alpha$1→4)GlcNAc- determinants (Fukuda, Biochim. Biophys. Acta 780:119-150 (1985)). Such modifications are significant because these determinants, which are present on neutrophils and monocytes, serve as ligands for E- and P-selectin present on endothelial cells and platelets, respectively (see, for example, Larsen et al., Cell 63:467-474 (1990)).

In addition, tumor cells often express a significant amount of sialyl $Le^x$ and/or sialyl $Le^a$ on their cell surfaces. The interaction between E-selectin or P-selectin and these cell surface carbohydrates may play a